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absence of lipid peroxidation, it is also possible that oxidized cytochrome *c* can inhibit lipid peroxidation by deviating the pathway of electron transfer. The effects of cytochrome *c* on lipid peroxidation are of interest because lipid peroxidation is a major mechanism of oxidative damage to membranes, and cytochrome *c* has been reported to have beneficial effects on tissues under damaging conditions (34). Furthermore, in a related study, a heme peptide from cytochrome *c* was recently reported to catalyze H_2O_2-mediated NADPH/NADH oxidation (35). Because the heme peptide was degraded by H_2O_2, it was suggested that the oxidation reaction may contribute toward intracellular protection of heme moieties. It was also of interest that when different fractions of PGB_x obtained during the preparative procedure (2) were tested, those fractions which were more active for conserving oxidative phosphorylation[5] were also more active in stimulating the oxidation of reduced cytochrome *c*.

The inhibitory effects of cytochrome *c* and PGB_x on lipid peroxidation are desirable for protecting tissues against oxidative damage. The interaction of cytochrome *c*, PGB_x, H_2O_2, and reductase which stimulates NADPH oxidation follows a complex mechanism containing both nonenzymatic and enzymatic components. More detailed studies will be necessary to resolve these two and determine their relationship to lipid peroxidation. However, at present, it can be speculated that PGB_x functions by removing the hydroperoxides. Since NADPH oxidation was stimulated by cytochrome *c* and PGB_x in the absence of lipid peroxidation, the possibility, that oxidized cytochrome *c* acts as an electron sink and PGB_x serves to keep cytochrome *c* oxidized must also be considered. In any case, if the prevention of membrane damage by inhibiting lipid peroxidation proves to be the mechanism whereby PGB_x protects mitochondria, the multiple effects of PGB_x (6, 7) on several membrane associated processes may become understandable.

[5] Personal communication from Herman W. Shmukler, Biochemistry Laboratory, Naval Air Development Center, Warminster, Pa.

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caused a small stimulation of NADPH oxidation, which was slightly enhanced by the addition of either cytochrome *c* and PGB_x, or H_2O_2. The stimulation due to the combined presence of cytochrome *c*, PGB_x, H_2O_2, and the reductase was greater than the sum of the individual stimulations. H_2O_2 also stimulated NADPH oxidation in the presence of cytochrome *c*; this effect was considerably enhanced by reductase (not shown in the table). EDTA when tested up to 5 mM had no effect. Qualitatively similar results were obtained using microsomes instead of the reductase.

DISCUSSION

The addition of PGB_x to reduced cytochrome *c* in the presence of H_2O_2 resulted in a striking stimulation of the oxidation of cytochrome *c*. Lack of stimulation by other prostaglandins or by EDTA or EDTA-Fe^{3+} indicated that the reaction was specific for PGB_x. However, the peroxide specificity was relatively low since the organic hydroperoxides tested (ethyl and t-butyl) also stimulated the oxidation. The oxidized cytochrome *c* could be reduced again either enzymatically or chemically, showing that its ability to undergo redox reactions was not impaired by PGB_x. In addition thermally induced transitions were preserved with transition temperatures and free energies of activation close to those reported (29) for the oxidation of cytochrome *c* by cytochrome oxidase in mitochondria.

Although the exact mechanism of interaction of the three reactants (PGB_x, peroxide, and cytochrome *c*) and the nature of the products of peroxide consumption were not clear, Scatchard analysis of the titration data suggested that one molecule of PGB_x probably binds to one molecule of oxidized cytochrome *c*. This binding may enhance the oxidizability of reduced cytochrome *c* as suggested by the similar dependence of the oxidation and the decrease in the Soret absorption on PGB_x concentration. The change in the Soret absorption is associated with an alteration of the heme region as indicated by the simplification of the CD spectrum, where the Soret was reduced to a single positive peak (405 nm). The disappearance of the negative Soret band in the CD spectrum of the H_2O_2-treated cytochrome *c*-PGB_x complex suggests that the conformation of the protein around the heme may resemble that of urea-treated cytochrome *c* which also showed only positive bands in this region (30). It was also reported (33) that when cytochrome *c* was treated with urea, the heme was more exposed and the redox potential became more negative. The similarity of the CD spectra of cytochrome *c* treated with urea or with PGB_x and H_2O_2 suggests that the PGB_x treatment may also lead to a more negative potential which would be compatible with the striking stimulation of the oxidation. The effect of temperature on the PGB_x-stimulated oxidation suggested that this nonenzymatic oxidation of cytochrome *c* resembled the enzymatic oxidation by cytochrome a_3 (29).

The similarity of the time courses of H_2O_2 depletion and cytochrome *c* oxidation suggests that peroxide was consumed in the reaction. Since oxygen release was not detected, catalase activity was probably not involved. Because (i) the organic hydroperoxides also stimulated cytochrome *c* oxidation and (ii) the stimulated rate decayed with time but (iii) was restored when the peroxide concentration was renewed, it is reasonable to expect that the hydroperoxides were also consumed in the reaction.

Lipid peroxidation is known to involve the production of semistable hydroperoxides from free radical intermediates which may be produced either by interaction of H_2O_2 with superoxide anion (O_2^-) or by the reaction of oxygen with unsaturated lipids. These hydroperoxides undergo homolytic scission to form free radicals which are then involved in autocatalysis of lipid peroxidation. Therefore, any reaction which can either remove or prevent the formation of H_2O_2 or the lipid hydroperoxides should inhibit lipid peroxidation. Microsomal lipid peroxidation was considerably (28%) inhibited by cytochrome *c* alone, and PGB_x enhanced this inhibition by 60%. Since cytochrome *c* stimulated NADPH oxidation via NADPH-cytochrome *c* reductase in the

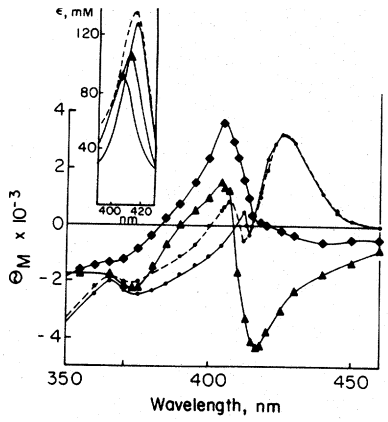

FIG. 5. Effect of PGB_x and H_2O_2 on the CD spectra of cytochrome *c*: The concentrations of cytochrome *c* and PGB_x were 0.12 and 0.42 mM. Insert: the corresponding changes in the absorption spectra. ● — ●, reduced cytochrome *c*; ● - - - ●, reduced cytochrome *c* + PGB_x; ▲, oxidized cytochrome *c*; ▲, cytochrome *c* + PGB_x + H_2O_2.

(1.4 μM) alone caused 28%. At this concentration of cytochrome *c*, the addition of 2 μg/ml PGB_x caused an additional 60% inhibition. Higher concentrations (>5 g/ml) of PGB_x were somewhat stimulatory. However, the peroxidation was decreased even at the higher PGB_x concentrations when cytochrome *c* was also present. Other prostaglandins tested which failed to stimulate cytochrome *c* oxidation also failed to inhibit lipid peroxidation.

Effects of PGB_x, Cytochrome c, and H_2O_2 on NADPH Oxidation

Lipid peroxidation presumably competes with other (drug metabolism) reactions for electrons donated by NADPH via a common microsomal electron transport pathway (31). Since the enzyme involved is NADPH-cytochrome *c* reductase (32), the effects of cytochrome *c*, PGB_x, and H_2O_2 on the oxidation of NADPH were examined in the absence of lipid peroxidation using the purified reductase. The results are summarized in Table II. The addition of cytochrome *c* alone or with PGB_x to NADPH caused no detectable oxidation, indicating that the cytochrome preparation (Type VI) was free of any oxidase activity. Cytochrome *c* and PGB_x stimulated NADPH oxidation provided H_2O_2 was added. The reductase preparation alone

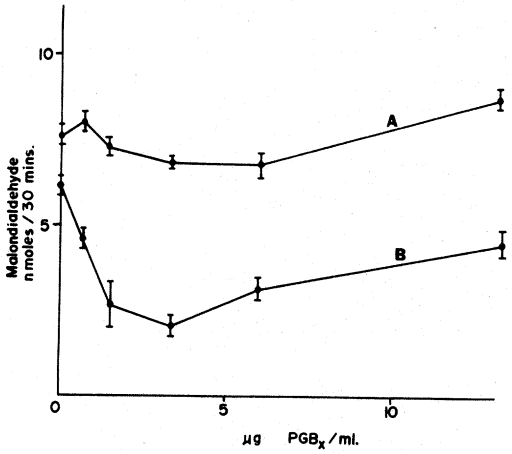

FIG. 6. Effect of PGB_x and cytochrome *c* on lipid peroxidation in microsomes: Thiobarbituric acid-positive products formed are expressed as nanomoles malondialdehyde. The assay system is described under Materials and Methods. Curve A, PGB_x alone; Curve B, PGB_x in the presence of 1.4 μM cytochrome *c*. Each point represents the average of two experiments.

TABLE II

EFFECTS OF CYTOCHROME *c*, PGB_x, AND H_2O_2 ON NADPH OXIDATION

Additions to NADPH	nmoles NADPH oxidized min^{-1}
None	0
Cytochrome *c*	0
PGB_x	0
H_2O_2	<0.5
cytochrome *c* + PGB_x	0
cytochrome *c* + PGB_x + H_2O_2 reductase	4.0
	2.4
Reductase + H_2O_2	3.6
Reductase + cytochrome *c* + PGB_x	3.6
Reductase + cytochrome *c* + PGB_x + H_2O_2	9.2

Note. The reaction medium was 0.1 M K-PO_4 (pH 7.3). Concentrations of reactants: NADPH (18 μM); H_2O_2 (0.176 mM); PGB_x (8.3 μM); cytochrome *c* (2.5 μM); and reductase (0.053 μM).

c, the rate of depletion of H_2O_2 was similar to the rate of cytochrome *c* oxidation, suggesting that H_2O_2 was consumed in the reaction. However, oxygen release could not be detected polarographically. When low concentrations of ethyl or t-butyl hydroperoxide were used the rate of oxidation was initially stimulated; this stimulation disappeared well before the cytochrome *c* was completely oxidized. The stimulated rate was recovered when the hydroperoxide concentration was renewed.

Fate of Cytochrome c

Figure 4 shows the effect of PGB_x on the absorption spectrum of oxidized cytochrome *c*. Addition of PGB_x to oxidized cytochrome *c* resulted in a shift of the Soret peak from 410 to 406 nm and a 20% decrease in absorptivity. The concentration of PGB_x required for half-maximum decrease in absorptivity of the oxidized cytochrome *c* (0.7 μM) was 6.8 μM, determined from a double-reciprocal plot which was linear within the concentration range of PGB_x tested. This concentration of PGB_x, although lower than the concentration required for half-maximum stimulation of the oxidation of cytochrome *c*, is reasonable because several equilibria are involved in the oxidation reaction.

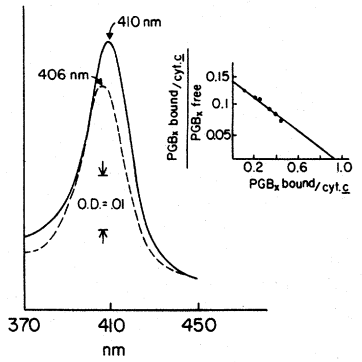

FIG. 4. The effect of PGB_x on the Soret absorption of cytochrome *c*: 0.66 μM cytochrome *c* in 0.05 M Hepes (pH 7.4) with no additions (—); with 5.8 μM PGB_x (- - -). The spectrum is corrected for the slight color contribution by PGB_x. Insert, Scatchard analysis of the titration.

Data obtained from the titration of oxidized cytochrome *c* with PGB_x were further analyzed assuming (i) the decrease in absorbance of oxidized cytochrome *c* represents binding of PGB_x to cytochrome *c*; (ii) cytochrome *c* has one binding site for PGB_x; (iii) the maximum decrease in absorptivity represents total cytochrome *c* present. The concentrations of PGB_x bound to cytochrome *c* and free in solution were calculated at each total PGB_x concentration. The Scatchard plot (Fig. 4) was linear with an intercept near 1.0. Although this intercept may be imposed by the assumptions made, the linearity of the plot suggests that they are correct.

Addition of PGB_x to reduced cytochrome *c* had little effect on the absorption spectrum in the α/β region, but shifted the Soret peak from 417 to 414 nm. After addition of H_2O_2 and completion of oxidation of the cytochrome *c*, the Soret peak was shifted to 406 nm, a position which could also be reached by adding PGB_x to oxidized cytochrome *c* (Fig. 4). The CD spectra (Fig. 5) of both oxidized and reduced cytochrome *c* agree well with those reported by Myer (30). The effect of adding PGB_x to reduced cytochrome *c* was on the secondary peak rather than the main Soret peak which remained at 425 nm. The secondary maximum was shifted from 412 to 409 nm with an increase in intensity much like the effect of urea on reduced cytochrome *c* (30). Addition of H_2O_2 shifted the Soret maximum to 405 nm. The resulting spectrum was identical to that when PGB_x and H_2O_2 were added to the oxidized cytochrome *c*. However, they differed from the spectrum of oxidized native cytochrome *c* in that the 418 nm minimum and 380–390 nm positive shoulder are missing.

Effects of PGB_x and Cytochrome c on Lipid Peroxidation

Figure 6 shows the effects of PGB_x in the presence and absence of cytochrome *c* on lipid peroxidation in microsomal membranes. Within the range tested, low concentrations of PGB_x (1–3.5 μg/ml) caused nearly 20% inhibition, while cytochrome *c*

At concentrations tested (up to 100 μg/ml), none of the prostaglandins tested stimulated cytochrome *c* oxidation nor did they have any effect on the PGB_x-dependent stimulation of oxidation. Certain metal complexes of EDTA, particularly EDTA-Fe^{3+}, stimulate lipid peroxidation (13, 26–28). However, neither EDTA (1 mM) nor EDTA-Fe^{3+} (25 M) stimulated the oxidation of cytochrome *c* even in the presence of H_2O_2.

Peroxide Specificity for Stimulation of Cytochrome c Oxidation

The effects of substituting ethyl or t-butyl hydroperoxide for H_2O_2 are summarized in Table I. At the concentrations of cytochrome *c* (0.7 μM) and PGB_x (2.8 μM) used in these experiments, the organic hydroperoxides were also effective at stimulating the oxidation.

Effect of Temperature on Cytochrome c Oxidation

The experiment shown in Fig. 1 was repeated at different temperatures. The results are shown in Fig. 2 in the form of an Arrhenius plot exhibiting a transition at 21.5°C. Apparent activation energies (ΔE) on the low- and high-temperature sides of the transition are 4.9 and 12 kcal mol^{-1}, respectively. These values agree well with the 3.9 and 15 kcal mol^{-1} reported for the oxidation of cytochrome *c* by cytochrome a_3 (29), which has a transition at 19°C.

TABLE I

OXIDATION OF REDUCED CYTOCHROME *c* BY PEROXIDES IN THE PRESENCE OF PGB_x

Peroxide	K^a (mM)	V_{max} (nmol/ml/min)
Hydrogen peroxide	0.4	2.0
Ethyl hydroperoxide	8.3	1.5
t-Butyl hydroperoxide	9.0	1.8

Note. The assay conditions were the same as for Fig. 1. The concentrations of PGB_x and cytochrome *c* were 2.8 and 0.7 μM, respectively.

[a] K = concentration of peroxide required for half-maximal stimulation. Calculated from the initial rates using the Linewaver-Burke equation.

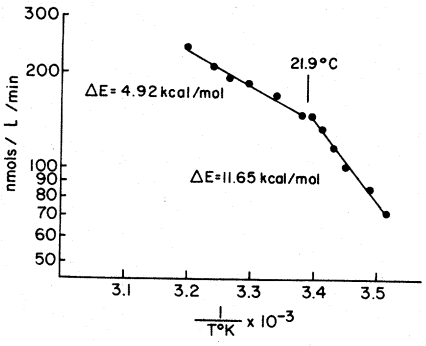

FIG. 2. Arrhenius plot of cytochrome *c* oxidation stimulated by PGB_x plus H_2O_2: The assay system is the same as for Fig. 1.

Fate of Peroxide

The time course of cytochrome *c* oxidation was correlated with that of peroxide depletion. Figure 3 shows that during the PGB_x-stimulated oxidation of cytochrome

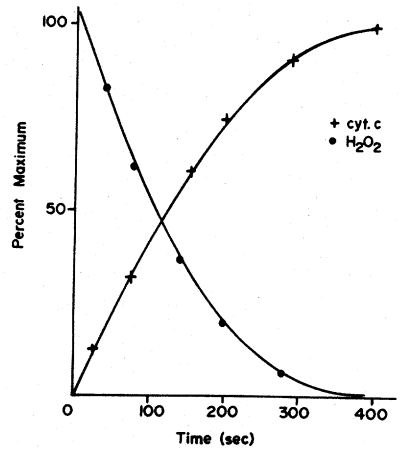

FIG. 3. Relationship between cytochrome *c* oxidation and H_2O_2 consumption. To 3.0 ml of 0.05 M Hepes, pH 7.4, equilibrated at 25°C, volumes of 10 μl or less were added to give the following concentrations: 0.125 mM H_2O_2; 1.39 μM PGB_x, and 1.1 μM chemically reduced cytochrome *c*. H_2O_2 and reduced cytochrome *c* were assayed simultaneously as described under Materials and Methods. Percentage maximum cytochrome *c* refers to the oxidized cytochrome *c* at a given time relative to the total reduced cytochrome *c* at zero time. For H_2O_2, the amount remaining is expressed as a percentage of the total consumed by PGB_x plus cytochrome *c* when all of the reduced cytochrome is oxidized.

determined with the appropriate concentration of PGB_x in the reference cuvette. Titration of oxidized cytochrome *c* with PGB_x was monitored as $\Delta A_{407-450\ nm}$ in the dual-wavelength filter photometer (the absorption at 450 nm was constant during the titration). The number of binding sites for PGB_x on cytochrome *c* was determined by Scatchard analysis of the titration data. Circular dichroism (CD) spectra were obtained using a Jasco 41C spectropolarimeter with 0.1- or 0.05-cm-pathlength cells.

Lipid peroxidation. Lipid peroxidation was initiated by adding 33 μM $FeCl_3$ and 0.1 ml of NADPH-generating system (to give a final concentration of 0.6 μM NADP + 3 mM glucose 6-phosphate + 0.2 unit/ml glucose-6-phosphate dehydrogenase) to glycylglycine buffer, pH 7.4, containing 2.7 mg of microsomal protein (total volume was 3.0 ml). Incubation was at 36°C. Formation of thiobarbituric acid-positive compounds, expressed as malondialdehyde, was used as an index of peroxidation (24).

NADPH oxidation. The effects of cytochrome *c* (Type VI), PGB_x, H_2O_2, and cytochrome *c* reductase[4] on NADPH oxidation in the absence of lipid peroxidation were studied in an assay system maintained at 25°C. The disappearance of NADPH was monitored fluorometrically (25) at 460 nm with an excitation wavelength of 340 nm using a Perkin-Elmer Model 650-10S spectrofluorometer. The decrease in fluorescence intensity as NADPH was oxidized was recorded on chart paper calibrated for a known concentration of NADPH, calculated from the absorption at 340 nm and an $E^{cm^{-1}}_{mM^{-1}}$ of 6.22.

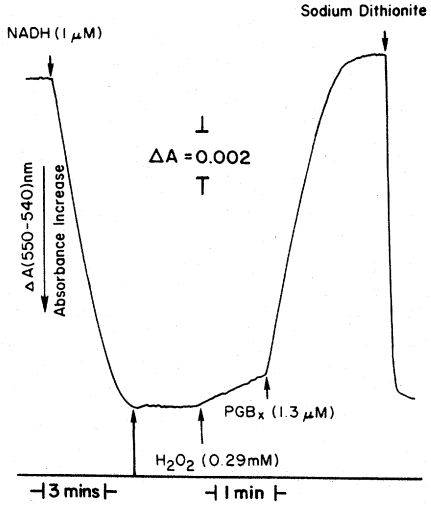

FIG. 1. The effects of H_2O_2 and PGB_x on the oxidation of cytochrome *c*: 3.0 ml of cytochrome *c* (0.07 μM) in 0.05 M Hepes (pH 7.4) at 25°C was reduced by the addition of 4 μg microsomal protein (the source of NADH-cytochrome *c* reductase), and 1.0 μM NADH. When reduction was complete, small volumes (10 μl or less) were added to give final concentrations of 0.29 mM H_2O_2 and 1.3 μM PGB_x. After completion of the oxidation, ferrocytochrome *c* was recovered by adding a few crystals of sodium dithionite. The reduction of cytochrome *c* was recorded at a chart speed of 3 min/in., and the oxidation at 1 min/in.

RESULTS

Stimulation of Oxidation of Cytochrome c by PGB_x and H_2O_2

Cytochrome *c* was reduced via the microsomal NADH-cytochrome *c* reductase system. In Fig. 1, the downward deflection of the trace indicates reduction and the upward deflection oxidation of cytochrome *c*. When reduction was complete, the addition of H_2O_2 followed by PGB_x resulted in a striking stimulation of the oxidation. Addition of either peroxide or PGB_x alone caused little stimulation. When the oxidation of the cytochrome *c* was complete, addition of sodium dithionite resulted in total recovery of reduced cytochrome *c*. At a constant concentration of H_2O_2, 8.9 μM PGB_x was required for half-maximum stimulation of the oxidation of cytochrome *c* (0.7 μM). Similarly at a constant concentration of PGB_x (2.8 μM), 0.4 mM H_2O_2 was required for half-maximum stimulation.

Because cytochrome *c* is only slightly oxidizable in the absence of enzymes, the experiment shown in Fig. 1 was repeated using chemically reduced cytochrome *c* to determine if any enzyme is involved in the PGB_x-dependent oxidation. The results were similar to those obtained when cytochrome *c* was reduced by the microsomal reductase system, indicating that no enzyme is required for the reaction.

Prostaglandin Specificity for the Oxidation of Cytochrome c

Several prostaglandins (PGE_1, PGE_2, $PGF_{2\alpha}$, $PGF_{2\beta}$, and 15-keto-PFG_2) were substituted for PGB_x in the reaction mixture to determine if stimulation of cytochrome *c* oxidation was specific to PGB_x.

[4] We thank Dr. M. J. Coon for the highly purified rabbit liver reductase.

inhibits reactions involving the F_1F_0-ATPase by blocking proton conduction through the F_0 moiety of the complex (6).

Cellular organelles including mitochondria (9) and microsomes (10) are effective H_2O_2 generators. Although H_2O_2 is a normal metabolite in cells (11), high concentrations can be toxic (12). The ischemic heart becomes more sensitive to H_2O_2 toxicity than the normal heart (12). The toxicity of H_2O_2 is probably due to its ability to generate hydroxyl radicals and other reactive oxygen species which can initiate chain reactions (13) that lead to the formation of lipid peroxides. In addition to being enzyme inhibitors, lipid peroxides affect membrane-bound enzymes and membrane premeability (14). Mitochondrial and microsomal membranes which contain a high degree of unsaturated lipids are especially susceptible to peroxidative damage (15). Several mechanisms exist which protect the cell against toxic products of oxygen metabolism. These include catalase, which removes H_2O_2, and glutathione-dependent enzymes, which prevent lipid peroxidation (16, 17).

To understand the mechanism whereby PGB_x protects mitochondria, we have studied its interaction with cytochrome *c*, a component of the respiratory chain. The prostaglandin stimulated the oxidation of cytochrome *c* in the presence of H_2O_2 or organic hydroperoxides. Because hydroperoxides formed by the reaction of oxygen with unsaturated lipids are involved in autocatalysis of lipid peroxidation (18), we investigated the effects of PGB_x plus cytochrome *c* on lipid peroxidation. The results of these studies are reported here.

MATERIALS AND METHODS

NADH, NADPH, and horse heart cytochrome *c* (Type III, except where noted) were obtained from Sigma Chemical Company. Sodium borohydride was used to chemically reduce cytochrome *c*; the borate formed was removed by gel filtration on Sephadex G-25. Concentrations of reduced cytochrome *c* were calculated using $E^{cm^{-1}}_{mM^{-1}}$ of 19.1 for $\Delta A_{540\text{-}550\ nm}$. Prostaglandins E_1, E_2, B_1, $F_{2\alpha}$, and $F_{2\beta}$ were gifts of UpJohn Company. PGB_x (preparation 25) which is a mixture of oligmers of PGB_1 (19, 20) was obtained from the Naval Air Development Center, Warminster, Pennsylvania. Concentrations of PGB_x were calculated assuming a molecular weight of 2400 (2, 6). Ethyl and t-butyl hydroperoxides were obtained from Polysciences and Wallis and Tiernan.

Bovine adrenocortical microsomes were prepared by a modification (21) of the method of Hogeboom *et al.* (22). The microsomes were suspended at a protein concentration of 20 mg/ml in buffered RNAse-free sucrose and stored at −70°C until used.

Redox reactions of cytochrome c. The redox reactions of cytochrome *c* were followed spectrophotometrically using a dual-wavelength filter photometer. A cuvette containing 3.0 ml of 0.05 M Hepes[3] (pH 7.4) was placed in the chamber of the photometer, equipped with a magnetic stirrer, and equilibrated at the appropriate temperature between 10 and 40°C as indicated in the legends to the figures. After obtaining a baseline of equal output of the two photomultipliers, small volumes (up to 10 μl) of the other reagents were added in the following order: cytochrome *c*, microsomes, NADH (1–2 μM, just sufficient to reduce the cytochrome *c* in the presence of microsomes), H_2O_2, and PGB_x to give the concentrations indicated in the legends to the figures. Changes in the absorption at 550 nm with respect to the isosbestic point at 540 nm ($\Delta A_{540\text{-}550\ nm}$) were recorded as a function of time. Reciprocal plots of the stimulated rate of cytochrome *c* oxidation versus the concentration of PGB_x or H_2O_2 were linear within the concentration ranges tested, and were used to calculate the concentrations of PGB_x and H_2O_2 required for half-maximal stimulation, and the maximum rates.

Correlation of cytochrome c oxidation and H_2O_2 consumption. After addition of H_2O_2 to the cytochrome *c* assay system and until all the reduced cytochrome *c* was oxidized, 5-μl aliquots were transferred at different times from the cytochrome *c* assay system in the dual-wavelength photometer to the H_2O_2 assay system. Hydrogen peroxide was assayed by the horseradish peroxidase (HRP)–scopoletin technique of Boveris *et al.* (23). The assay system consisted of 0.8 μM HRP, 6.5 μM scopoletin, 1 mM EDTA, and 0.05 M potassium phosphate (pH 7.0) in a total volume of 3.0 ml equilibrated at 25°C in a cuvette placed in a spectrofluorometer with the excitation wavelength set at 365 nm and emission at 450 nm. The decrease in emission was recorded on chart paper which had been calibrated for the emission of a known concentration of scopoletin. The peroxide remaining was calculated from the fluorescence.

Spectrophotometric measurements. Absolute absorption spectra were recorded with a Perkin-Elmer Model 571 spectrophotometer. The effect of PGB_x on the absorption spectrum of oxidized cytochrome *c* was

[3] Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Interaction of PGB_x and Peroxides with Cytochrome *c* and Inhibition of Lipid Peroxidation[1]

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PGB_x, a derivative of prostaglandin B_1, stimulated the oxidation of cytochrome *c* in the presence of H_2O_2. Although the reaction was nonenzymatic, the apparent activation energies of 12 and 4.9 kcal above and below the transition at 21.5°C were similar to those for oxidation by cytochrome oxidase. Depletion of H_2O_2 and oxidation of cytochrome *c* followed similar time courses, suggesting that H_2O_2 was consumed in the reaction. PGB_x was a specific requirement, but organic hydroperoxides (ethyl and T-butyl) could replace H_2O_2. Low concentrations of ethyl or t-butyl hydroperoxide initially stimulated the oxidation of cytochrome *c;* this stimulation disappeared before completion of the oxidation, but was restored when the hydroperoxide concentration was renewed, suggesting that these hydroperoxides were probably also consumed in the reaction. The concentration of PGB_x (8.9 μM) required for half-maximum stimulation of the oxidation was similar to the apparent K_d for its dissociation from oxidized cytochrome *c* (6.8 μM). Binding data and CD spectra suggested that a 1:1 complex between cytochrome *c* and PGB_x was formed, altering the conformation of the heme region. This conformational change caused a shift of the Soret absorption peak from 410 to 406 nm and may be responsible for the enhanced oxidizability of the cytochrome *c* by H_2O_2. Cytochrome *c* inhibited lipid peroxidation in microsomes, an effect enhanced by the addition of PGB_x. In the absence of lipid peroxidation, cytochrome *c* and PGB_x stimulated NADPH oxidation via NADPH–cytochrome *c* reductase. Thus the inhibition of lipid peroxidation by cytochrome *c* and PGB_x may involve either the removal of hydroperoxides or deviation of electron transfer away from the pathway for lipid peroxidation.

PGB_x is a water soluble oligomeric derivative of prostaglandin B_1 (1), reported to have a protective effect against anoxic-ischemic stress caused by coronary ligation (2) as well as on degenerative changes in mitochondria from infarcted areas of the fibrillating heart (3, 4). In the perfused rat heart, PGB_x influences metabolite concentrations and mechanical activity (5). It also possesses a unique property of conserving oxidative phosphorylation in isolated aged mitochondria (1, 2). The protective effect on mitochondria appears to be concentration dependent; above the protective level PGB_x inhibits oxidative phosphorylation (6). It is of interest that at the levels (2–8 μM) which protected the aged mitochondria, PGB_x was without effect on normal mitochondria (2). Although their relevance for the protection of mitochondria is not known, several reactions of PGB_x have been described. The prostaglandin derivative has Ca^{2+} ionophoretic activity in sarcoplasmic reticulum, bovine heart mitochondria, and liposomes (7, 8). In addition, it

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